

Animal Conservation

Volume 16 | October 2013 | Number 5



The rapid publication journal for quantitative studies in conservation

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FEATURE PAPER

Nest depth may not compensate for sex ratio skews caused by climate change in turtles

J. M. Refsnider, B. L. Bodensteiner, J. L. Reneker & F. J. Janzen

Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA, USA

Keywords

Chrysemys picta; incubation temperature; nest-site choice; painted turtle; performance.

Correspondence

Jeanine M. Refsnider, Department of Environmental Science, Policy, and Management, University of California, Berkeley, 130 Mulford Hall, Berkeley, CA 94720-3114, USA.
Tel: +1 510 643 7430; Fax: +1 510 643 5438
Email: refsneider@berkeley.edu

Editor: Jeff Johnson
Associate Editor: Christopher Raxworthy

Received 7 May 2012; accepted 30 January 2013

doi:10.1111/acv.12034

Abstract

Maternal ability to match nest characteristics with environmental conditions can influence offspring survival and quality, and may provide a mechanism by which animals can keep pace with climate change. In species with temperature-dependent sex determination that construct subterranean nests, the depth of the nest may affect incubation temperatures, and thus offspring sex ratio. Maternal adjustment of nest depth may be a mechanism by which climate change-induced sex ratio skews could be prevented in globally imperiled taxa such as turtles. We experimentally manipulated nest depth within a biologically relevant range in nests of the model turtle species *Chrysemys picta*. We then quantified the effects of nest depth on incubation regime, offspring sex ratio and offspring performance. We found no effect of nest depth on six parameters of incubation regime, nor on resultant offspring survival, size or sex ratio. However, deeper nests produced hatchlings that weighed less, and were faster at righting themselves and swimming, than hatchlings from shallower nests. We suggest that cues used by females in adjusting nest depth are unreliable as predictors of future incubation conditions, and the adjustment in nest depth required to affect sex ratio in this species may be too great to keep pace with climate change. Therefore, maternal adjustment of nest depth seems unlikely to compensate for climate change-induced sex ratio skews in small-bodied, freshwater turtles.

Introduction

Human activities are contributing significantly to global climate change, one result of which is a predicted increase in global temperatures of 1.1–6.4°C by 2100 (Solomon *et al.*, 2007). A temperature increase of this magnitude is likely to have dramatic effects on species and ecosystems, but many of these outcomes are difficult to predict because they involve indirect effects of environmental changes on a wide variety of taxa and occur via complex pathways (Barnosky *et al.*, 2012). Some thermally sensitive traits that are directly impacted by climate can have demographic consequences for populations. One example is temperature-dependent sex determination (TSD), in which offspring sex is irreversibly determined by the temperature experienced by developing embryos (Bull, 1980). TSD occurs in many reptile groups as well as some fishes and invertebrates (Bergerard, 1972; Valenzuela & Lance, 2004). In reptiles with TSD, the temperature range within which the complement of offspring sex within a clutch shifts from all of one sex to all of the other sex is generally narrow, and is often less than 1°C (Ewert, Jackson & Nelson, 1994). Consequently, population sex ratios are extraordinarily sensitive to temperature changes because a small shift in environmental temperature

could dramatically alter offspring sex ratio (e.g. Schwanz *et al.*, 2010). Human-induced climate change could severely impact reptiles with TSD by resulting in populations comprised of predominantly one sex (Janzen, 1994a; Mitchell *et al.*, 2008), and therefore recognizing the potential effects of climate change on species with TSD is important if conservation efforts are to be effective.

Maternal nest-site choice and nest construction influence offspring survival and quality, as well as sex in species with TSD (reviewed in Refsnider & Janzen, 2010). For example, in birds, the amount of vegetative cover around a nest can reduce its visibility to predators (e.g. Martin & Roper, 1988; Stokes & Boersma, 1998), and the direction of a nest's opening may protect offspring from thermal stress (e.g. Walsberg & King, 1978; Hartman & Oring, 2003); in reptiles, the temperature within a nest can affect offspring performance (e.g. Miller, Packard & Packard, 1987; Van Damme *et al.*, 1992; Shine *et al.*, 1997). Maternal ability to match nest location and construction to environmental conditions is an important determinant of a female's reproductive success, and may be particularly critical in allowing females to shift nest characteristics to match changing environmental conditions and thereby continue to successfully reproduce. Matching nest characteristics with

prevailing environmental conditions could occur via two, non-mutually exclusive mechanisms: microevolutionary responses of populations over several generations, or plastic responses among or even within individuals (Bulmer & Bull, 1982). The importance of both mechanisms in population responses to inter-annual climatic variation has been demonstrated in species with TSD (Morjan, 2003a; McGaugh *et al.*, 2010; Refsnider & Janzen, 2012), but the relative importance of each mechanism is dependent upon the component of nest-site choice being examined. In reptiles with TSD, components of nest-site choice that could theoretically change in response to environmental conditions include date of nesting, shade cover over the nest site, nest microhabitat such as soil moisture and depth of the nest cavity (e.g. Morjan, 2003a; Doody *et al.*, 2006a; Schwanz & Janzen, 2008). For example, in painted turtles, shade cover over the nest predicts sex ratio (Janzen, 1994b), and behavioral plasticity in maternal selection of shade cover over the nest appears to allow female turtles to match nest microhabitat to prevailing environmental conditions (Refsnider & Janzen, 2012). However, heritability in choice of shade cover appears insufficient to compensate for inter-annual climatic variations (McGaugh *et al.*, 2010). Similarly, although nesting date is a plastic response based on the preceding winter's climate, it is not repeatable within individuals and therefore is unlikely to respond to selection (Schwanz & Janzen, 2008). Therefore, in both the shade cover and phenological components of nest-site choice in painted turtles, behavioral plasticity was a more important mechanism than microevolutionary changes in population responses to inter-annual climate variation.

Nest depth is another component of nest-site choice that could vary in response to prevailing environmental conditions, and thereby match incubation regime with climate. Modeling studies suggest that nest depth affects both incubation temperature and the magnitude of temperature variation (Georges, Limpus & Stoutjesdijk, 1994), and empirical data show that incubation temperature differs with nest depth in turtles (Roosenburg, 1996), lizards (Shine & Harlow, 1996) and crocodylians (Leslie & Spotila, 2001). Indeed, altering nest depth as global temperatures increase may be critical for some species; for example, tuatara (the sole representative of an ancient reptilian order) are predicted to produce all male offspring at current nest depths under maximum climate-warming scenarios (Mitchell *et al.*, 2008). The mechanism (i.e. microevolutionary response or behavioral plasticity) behind such adjustments in nest depth have not been studied. For example, nests constructed at depths that were previously representative of the population average could, under severe climate-warming scenarios, experience lethally high temperatures, which would select for deeper, cooler nests and result in a microevolutionary change in mean population nest depth. Alternatively, if nest depth is behaviorally plastic, individual females might base nesting behavior on ambient temperatures during the period from spring emergence and onset of nesting, and construct deeper nests in warm years and more superficial nests in cool years.

Adjusting nest depth may not be a component of nest-site choice that will compensate for climate change in all cases, and therefore, it is important to understand the circumstances under which nest depth adjustment may or may not be possible. For example, in some reptiles, nest depth is not related to nest temperature (eastern fence lizard; Warner & Andrews, 2002), does not contribute to variation in sex ratio (freshwater turtles; Vogt & Bull, 1982) or cannot be altered because of low availability of nest sites with suitable microhabitats, such as appropriate soil type (tuatara; Mitchell *et al.*, 2008). In other reptiles, despite climatic differences among geographically widespread populations, nest depths do not differ among populations (Australian water dragon; Doody *et al.*, 2006a) or, when they do differ, the differences may be a function of female body size (painted turtle; Morjan, 2003b) rather than adaptations to local climate. Finally, even when nest depth changes in response to warming temperatures, such change may be insufficient to compensate for the magnitude of climatic warming (three-lined skink; Telemeco, Elphick & Shine, 2009). The preceding studies demonstrate that testing whether adjustment of nest depth could compensate for climate change-induced sex ratio skews is an important part of understanding how reptiles, which tend to be long lived, may respond to rapid environmental change. One additional note is that incubation conditions within a nest site affect numerous offspring traits in species with TSD other than sex, including size (Brown & Shine, 2004), growth rate (Brooks *et al.*, 1991), metabolism (Van Damme *et al.*, 1992), speed (Miller, 1993) and predator avoidance behavior (Burger, 1989). Because shifts in maternal nesting behavior in response to climate change may have simultaneous and perhaps unexpected effects on offspring size and performance (Refsnider, 2013), it is important to incorporate measures of offspring quality as well as sex ratio in studies on species that are particularly temperature sensitive.

We conducted a manipulative experiment to test the hypothesis that incubation temperature in naturally constructed turtle nests decreases with depth, which would indicate that adjustment of nest depth might compensate for inter-annual variation in climate. Our study is a first step toward evaluating the capacity of nest depth adjustment to compensate for inter-annual climatic variation. Although this study does not directly address the mechanism (i.e. microevolutionary response or behavioral plasticity) behind potential changes in nest depth in response to inter-annual climatic variation, we discuss the support our results provide for potential underlying mechanisms. Instead, we were interested in quantifying the effects of nest depth adjustment on offspring sex and performance, regardless of how such adjustment might occur, to determine whether this component of nest-site choice has any potential to compensate for inter-annual climatic variation, and by extension, for climate change. Because our study design required the euthanasia of a portion of hatchlings produced at the study site, we used a common species as a model to avoid negatively impacting any populations of rarer species. Nevertheless, our results should be of use to managers involved in

conservation efforts for similar, imperiled turtle species. This study had two primary objectives. First, we used a long-term dataset to determine whether mean nest depth in a wild turtle population was correlated with annual climate. Second, we conducted a manipulative experiment to determine the effect of nest depth on thermal characteristics of the incubation environment within the nest cavity (hereafter incubation regime), offspring sex ratio and offspring performance.

We conducted this experiment using the western painted turtle, *Chrysemys picta bellii*, a small-bodied freshwater turtle in the family Emydidae. The painted turtle is widely dispersed across the USA and southern Canada, and the western subspecies occurs primarily west of the Mississippi River. Painted turtles inhabit a variety of aquatic habitats including rivers, lakes and ponds. In early summer, females emerge from wetlands to nest in open areas such as beaches and lawns. Incubation lasts ~55–85 days depending on temperature (Ernst, 1971; F. Janzen, unpublished data). After hatching, neonates remain in the nest cavity through their first winter and emerge the spring following nest construction, at which time they travel terrestrially until reaching a wetland habitat (e.g. Paukstis, Shuman & Janzen, 1989).

Materials and methods

Long-term climate and nest depth trends

Our study site was a nesting beach on the northeastern side of a 1.5-ha island in the Mississippi River in Carroll County, Illinois, USA, at the Thomson Causeway Recreation Area. Data collection for the long-term study of nesting ecology is described in Schwanz *et al.* (2010). Briefly, we patrolled a nesting area in the South Potter's Marsh Campground hourly between 06:00 and 21:00 h from mid-May through early July. We observed nesting turtles from a distance until they completed the nesting process, at which time we briefly captured females for individual identification before releasing them. As in Schwanz *et al.* (2010), we excavated nests within 24 hours of construction to assess clutch size, egg mass and nest depth; we then re-covered nests with soil and left them to incubate *in situ*.

Nest depths were measured, using a straight-edge ruler, as the vertical distance from the soil surface to the base of the nesting cavity. We recorded depths of 2371 nests from 2000 to 2010, and we used these data to determine the mean population nest depth in each year. We focused on the grand mean of population nest depth from 2004 to 2009 ($n = 1126$) to determine our experimental treatments (see below) because turtles nesting in 2004–2010 are likely from the same age cohort. We used mean air temperature in May, acquired from the National Climate Data Center (<http://www.ncdc.noaa.gov>) for nearby Clinton, IA, as an indicator of climatic conditions in each year from 2000 to 2010.

Nest depth manipulation experiment

We conducted a nest depth manipulation experiment on a subset of painted turtle nests constructed in the North

Potter's Marsh Campground in June 2010. Nests were located and processed as described above. However, in this experiment, we randomly assigned 44 nests to one of three nest depth treatments: shallow ($n = 14$), mean ($n = 15$) or deep ($n = 15$). The mean treatment was 8.7 cm, which was the grand mean nest depth for 1126 nests constructed during 2004–2009. The shallow and deep treatments were 6.7 and 10.7 cm, respectively, are equivalent to two standard deviations from the grand mean, and were selected to represent biologically relevant values that, while relatively extreme compared to most nests, were not outside the range of physically possible nest depths in this population. To achieve the assigned nest depth treatment, we either added soil to the bottom of the nest cavity (to decrease nest depth) or excavated additional soil (to increase nest depth) before replacing the eggs. In cases where the entire clutch would no longer fit in a nest because of artificial decreasing of the nest depth, we increased the size of the egg chamber horizontally (rather than vertically) until all eggs would fit. In addition to manipulating nest depths, we inserted a temperature logger (iButton, Embedded Data Systems, Lawrenceburg, KY, USA) among the eggs in the center of each nest. Loggers recorded nest temperatures hourly throughout incubation. Also, because canopy cover over nests affects incubation temperature (Morjan & Janzen, 2003), we took a hemispherical photograph over each nest and quantified canopy cover using Gap Light Analysis software (Frazer, Canham & Lertzman, 1999 as in Doody *et al.*, 2006b). Finally, to minimize nest loss because of predation (which can range up to 95% of nests; Schwanz *et al.*, 2010), we covered all nests with a 10-cm² piece of wire mesh staked at each corner.

We retrieved temperature loggers and all surviving hatchlings in September 2010 (after hatching but before nest emergence). For each nest, we considered the incubation period to start on day 0 (the day of oviposition) and continue through day 70. For reptiles with TSD, the thermosensitive period is generally the middle third of embryonic development (Wibbels, Bull & Crews, 1994). As we did not directly observe when hatching occurred in any nest, we considered days 16–45 to encompass the thermosensitive period during which sex differentiation occurs. We then calculated six parameters related to incubation conditions for each nest (hereafter incubation regime): minimum and maximum incubation temperatures (the lowest and highest temperature recorded during the 70-day incubation period), mean temperature throughout the incubation period (days 0–70) and the thermosensitive period (days 16–45) and the mean daily range (for each 24-hour period, highest recorded temperature – lowest recorded temperature) for the incubation period and the thermosensitive period.

Offspring quality and sex ratio

After retrieving hatchlings, we calculated the survival rate of each nest as the number of live hatchlings retrieved, divided by the known clutch size. We carefully cleaned and dried hatchlings, and recorded their plastrons with a color scanner to facilitate individual identification. We then weighed and

measured (straight carapace length) all hatchlings and housed clutchmates together in plastic deli cups containing moist soil over the winter in an incubator (Revco, Thermo Scientific, Asheville, NC, USA) at 4°C, conditions comparable to overwintering temperatures observed in wild nests from the Illinois population (Weisrock & Janzen, 1999). Starting in mid-March 2010, we gradually increased incubator temperature to 19°C over a 2-week period and kept hatchlings at 19°C thereafter.

We conducted three performance tests on each hatchling: (1) righting time (the time it took for a hatchling placed on its back to right itself); (2) sprinting time (the time it took a hatchling to walk 0.5 m); and (3) swimming time (the time it took for a hatchling to swim 1.0 m). The performance tests used here simulated a hatchling's journey from the nest site to wetland habitat, and then to suitable habitat within the wetland, following nest emergence in the spring. Therefore, we tested each hatchling in the order of righting time, immediately followed by sprinting time, and swimming time immediately following sprinting time. All hatchlings underwent two trials of three performance tests each, with the two trials separated by *c.* 2 weeks. In all three performance tests, we timed a hatchling's latency to begin moving (latency), the total time taken to complete the test from initial placement of the hatchling until the test was completed (total) and the time during which the hatchling was actively moving during completion of the test (active, or total – latency). We recorded all times to the nearest second using a digital stopwatch, and censored all tests at 180 s. During the performance tests, researchers were blind to the depth treatment of the nest from which hatchlings were produced.

Upon completion of the performance trials, we euthanized a subset of the hatchlings by a pericardial overdose of 0.5 mL of 1:1 sodium pentobarbital : water. To avoid negatively impacting the population, we euthanized and sexed up to six hatchlings per nest. Because most (66%) nests at our study site are unisexual (Janzen, 1994b), the sex ratio of a nest can be estimated by determining sex in a portion of the hatchlings from that nest (Schwanz *et al.*, 2010), which reduces the number of individuals that must be euthanized. We assigned sex based on macroscopic examination of the gonads (Schwarzkopf & Brooks, 1985), classifying individuals lacking oviducts and possessing short gonads as males and those with complete oviducts and long gonads as females. After sexing, we preserved all specimens in 70% ethanol; the remaining hatchlings that were not sexed were released at the collection site in May 2011.

Statistical analyses

We conducted all statistical analyses in SAS 9.2 (SAS Institute, Cary, NC, USA). We first analyzed relationships between nest depth, female body size, year and annual climate using general linear regression. We then compared each of the six incubation regime parameters among the three nest depth treatments using one-way analysis of covariance (ANCOVA) in the General Linear Model (GLM) Procedure with canopy cover as a covariate. We tested for

differences among nest depth treatments in mean hatchling mass and carapace length using one-way ANCOVA in the MIXED procedure with canopy cover and mean initial egg mass as covariates, and we tested for differences among treatments in per cent hatching success using one-way ANCOVA in the GLM Procedure with canopy cover as a covariate. Differences in nest sex ratios among nest depth treatments were compared using a chi-square goodness-of-fit test (Wilson & Hardy, 2002). Because shade cover predicts sex ratio in natural nests at the study site (Janzen, 1994b), we also assessed whether shade cover continued to predict sex ratio of experimentally manipulated nests. This was done using logistic regression in the GENMOD procedure, with depth treatment and shade cover as independent predictors. Finally, we analyzed differences among treatments in median hatchling performance (i.e. latency, active and total median times for righting, sprinting and swimming) using Kruskal–Wallis tests in the NPAR1WAY procedure, and corrected the *P*-values for multiple tests ($m = 9$) using the Bonferroni adjustment.

Results

We analyzed the total depth of 2371 unmanipulated painted turtle nests from 2000 to 2010 (Fig. 1). Mean May temperature did not significantly increase over time between 2000 and 2010 at the study site ($P = 0.96$; $R^2 = 0.0003$; Fig. 2a). Mean annual nest depth ranged from 8.48 to 9.11 cm, and there was a slight but statistically significant positive correlation between nest depth and mean May air temperature ($F_{1,2369} = 19.58$, $R^2 = 0.008$, $P < 0.0001$; Fig. 2b). Mean annual female body size decreased over time ($F_{1,9} = 7.73$, $R^2 = 0.46$, $P = 0.02$; Fig. 2c), but was not correlated with mean annual nest depth ($P = 0.88$).

In 2010, we manipulated depths in 44 nests. Flooding of the Mississippi River in August caused the complete loss of 23 nests; therefore, we have incubation regime data for 8 shallow, 9 mean and 4 deep nests. The hatchlings in one additional nest were crushed by construction machinery shortly before we retrieved hatchlings from the nests, but because we were able to determine the sex of the crushed

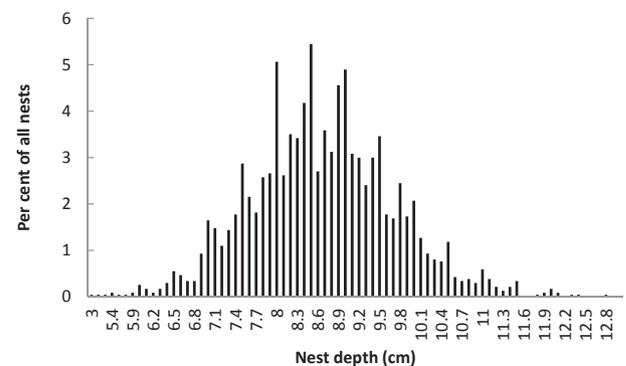


Figure 1 Frequency distribution of nest depth in 2371 painted turtle (*Chrysemys picta*) nests at Thomson Causeway Recreation Area, Carroll County, Illinois from 2000 to 2010.

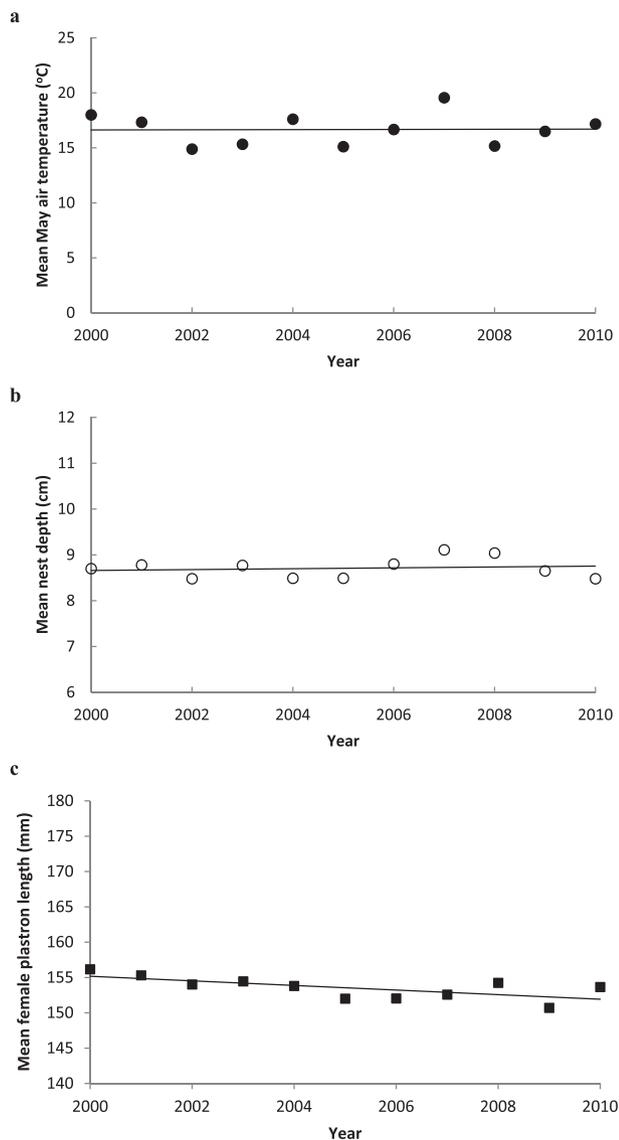


Figure 2 Mean air temperature in May (a), mean nest depth (b) and mean body size of nesting female (c) painted turtles (*Chrysemys picta*) at Thomson Causeway Recreation Area, Carroll County, Illinois from 2000 to 2010. Nest depth was positively correlated with mean May temperature ($F_{1,2369} = 19.58$, $R^2 = 0.008$, $P < 0.0001$), but mean May temperature did not significantly increase from 2000 to 2010 at the study site ($R^2 = 0.0003$). Annual mean female body size decreased over time ($F_{1,9} = 7.73$, $R^2 = 0.46$, $P = 0.02$), but was not correlated with annual mean nest depth ($P = 0.88$).

hatchlings, they were included in the analysis of sex ratio differences among treatments. We retrieved 185 live hatchlings from the 20 surviving nests, and 165 of these hatchlings survived the overwintering period to be included in the performance tests.

None of the six parameters of incubation regime differed among nest depth treatments (all P -values > 0.10 ; Table 1). Shade cover did not differ over nests assigned to the three depth treatments ($F_{2,17} = 0.20$, $P = 0.82$), but it was a mar-

ginally significant predictor of nest sex ratio ($\chi^2 = 3.3$; 1 d.f.; $P = 0.07$; Table 2). Nest depth treatment did not influence per cent hatching success ($F_{3,15} = 1.47$; $P = 0.26$), mean hatchling carapace length ($F_{2,135} = 0.90$; $P = 0.41$) or offspring sex ratio ($\chi^2 = 6.3$; 2 d.f.; $P = 0.10$; Table 2). In contrast, nest depth was negatively correlated with hatchling mass ($F_{4,148} = 17.15$; $r = 0.56$; $P = 0.04$). Hatchlings from the three nest depth treatments did not differ in any measure of median sprinting time (all P -values > 0.05). However, hatchlings from deeper nests had shorter total righting times ($\chi^2 = 13.0$; 2 d.f.; $P = 0.01$), active righting times ($\chi^2 = 16.6$; 2 d.f.; $P = 0.002$), latency to swim times ($\chi^2 = 20.8$; 2 d.f.; $P = 0.001$) and total swimming times ($\chi^2 = 13.6$; 2 d.f.; $P = 0.01$; Fig. 3) than hatchlings from shallower nests.

Discussion

The strong influence of nest-site characteristics on offspring survival, quality and phenotype illustrates the importance of maternal ability to match nest location and characteristics with environmental characteristics. Matching nest-site characteristics to prevailing environmental conditions may be either behaviorally plastic or an evolved response, both of which are mechanisms by which organisms could track climate change and thereby mitigate some negative impacts, such as skews in sex ratios in species with TSD. One nest-site characteristic that is potentially adjustable to match environmental conditions is nest depth, whereby females may simply construct deeper nests in warmer years. To determine the effect of nest depth on incubation regime, offspring sex ratio and offspring performance, we experimentally manipulated depths of naturally constructed nests within a biologically relevant range in a model turtle species with TSD.

May air temperatures at our study site displayed no consistent trend over the course of the 11-year study, yet nests were deeper in years with warmer May temperatures. Similarly, *C. picta* nests in climatically warmer New Mexico are slightly deeper than *C. picta* nests at our cooler Illinois site (Morjan, 2003b). While population differences in nest depths might be an evolved response to climatic differences, the fact that mean nest depths in Illinois tracked air temperatures during the nesting season suggests behavioral plasticity in females' ability to adjust nest depth based on prevailing environmental conditions. Similarly, the study population is known to adjust timing of nesting in response to conditions during the previous winter (Schwanz & Janzen, 2008). Importantly, however, cues associated with past or current conditions might not be predictive of future incubation conditions. In our study, despite the fact that nest depth tracked temperatures during the May nesting season, May temperatures were not correlated with temperatures in July ($P = 0.58$), which is the approximate thermosensitive period at the study site (Janzen, 1994a). Therefore, air temperature at the time of nesting might be an unreliable indicator of the incubation regime a nest site will experience during the thermosensitive period, unless climatic conditions follow a predictable, linear trend and do

Table 1 Mean values for six parameters of incubation regime in manipulated painted turtle (*Chrysemys picta*) nests at Thomson Causeway Recreation Area, Carroll County, Illinois in 2010

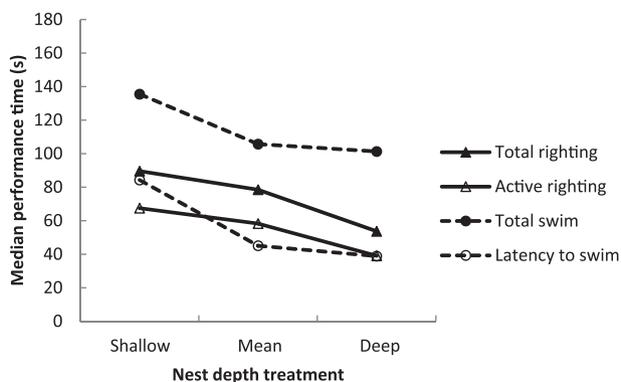
	Shallow (6.7 cm) (n = 8 nests)	Mean (8.7 cm) (n = 9 nests)	Deep (10.7 cm) (n = 4 nests)
Minimum incubation temp	15.6 ± 1.3	17.1 ± 1.6	17.1 ± 1.4
Maximum incubation temp	38.2 ± 4.6	37.8 ± 3.4	36.8 ± 4.5
Incubation temp	26.1 ± 1.7	25.9 ± 1.0	25.7 ± 1.3
Daily range of temp, incubation period	8.0 ± 0.7	7.4 ± 1.1	7.0 ± 1.0
Daily range of temp, thermosensitive period	8.7 ± 0.9	7.6 ± 1.5	7.5 ± 1.2

Values shown are means (°C) ± one standard deviation (number of hatchlings included in analysis). None of these parameters differed among nest depth treatments. All flooded nests are excluded.

Table 2 Mean hatchling survival, hatchling mass, hatchling carapace length (CL), sex ratio and shade cover in manipulated painted turtle (*Chrysemys picta*) nests at Thomson Causeway Recreation Area, Carroll County, Illinois in 2010

	Shallow (6.7 cm) (n = 8 nests)	Mean (8.7 cm) (n = 9 nests)	Deep (10.7 cm) (n = 4 nests)
% hatchling survival	76 ± 22 (75)	79 ± 15 (67)	92 ± 6 (43)
Hatchling mass (g)	4.3 ± 0.6 (75)	4.1 ± 0.6 (67)	4.0 ± 0.6 (43)
Hatchling CL (mm)	25.7 ± 1.5 (75)	25.2 ± 1.5 (67)	25.4 ± 1.4 (43)
% male	22 ± 25 (76)	8 ± 10 (67)	30 ± 21 (53)
% shade cover over nest	48.2 ± 6.6	48.0 ± 12.4	50.2 ± 10.2

Values shown are means ± one standard deviation (number of hatchlings included in analysis); boldface indicates significant differences. All flooded nests are excluded.

**Figure 3** Nest medians for total righting, active righting, total swim and latency to swim time of hatchling painted turtles (*Chrysemys picta*) from nests of three different depth treatments at Thomson Causeway Recreation Area, Carroll County, Illinois in 2010. Deeper nests produced hatchlings that were faster to right themselves and to swim.

not simply vary unpredictably among years. If nest depth is adjusted based on current conditions that are not necessarily predictive of future incubation conditions, then nest depth adjustment, even if it is behaviorally plastic, might not be a component of nest-site choice that could reliably compensate for climate change. Female turtles might instead use a more reliable cue to predict incubation conditions during the thermosensitive period, such as shade cover (Janzen, 1994b). Our study provides additional support for the importance of shade cover as a predictor of future incubation conditions in that, even after our manipulation of nest depth, shade cover was a stronger predictor of offspring sex ratio than nest depth.

Within the biologically realistic range of nest depths tested here, incubation regime did not differ among nest depth treatments. It is not surprising, then, that we observed no significant difference in the thermally sensitive trait of sex ratio among our nest depth treatments. There were also no differences in hatchling survival or carapace length among depth treatments, although hatchling mass declined as nest depth increased. The lack of difference in sex ratio among treatments suggests that, while nest depths may be adjusted slightly under thermally divergent climatic conditions, the adjustment is of insufficient magnitude to affect offspring sex ratio in this small-bodied species. Therefore, for nest depth to compensate for potential sex ratio skews produced by climate change in this species, females at the Illinois site would have to construct nests that are 2 cm deeper than the current population mean, which translates into depths greater than two standard deviations from the current population mean. Because maximum nest depth in turtles is constrained by female limb length (Tiwari & Bjorndal, 2000; Refsnider, 2012), selection for increased nest depth would likely necessitate a concomitant increase in female body size if females are to construct considerably deeper nests. Strong direct selection on adult survival can cause rapid shifts in reptile body size (e.g. Wolak *et al.*, 2010), but indirect selection for increased female body size to construct deeper nests is likely much weaker; therefore, compensation for rapid climate change through selection for deeper nests is likely to be evolutionarily constrained by relatively weak selection for increased female size (Refsnider, 2012). Female body size does not appear to be a strong driver of nest depth in the study population for two reasons. First, female turtles are currently constructing nests farther from their maximum physical capacity than populations at the edge of the species'

geographic range (Refsnider, 2012). Second, changes in mean nest depth over time were not correlated with mean body size of reproductive females. However, populations at both the northern and southern edges of the species' range are currently constructing nests near their maximum physical capacity (Refsnider, 2012), suggesting that an adjustment in nest depth to compensate for climate change would require an increase in female body size in at least some populations.

The large shift in nest depth that would be required to affect incubation regimes sufficiently to alter sex ratios in this species seems unlikely to occur in response to climatic warming for the reasons discussed above. Similarly, in a reptile where nest depth was not constrained by female size and females did adjust nest depth to match climatic conditions, the adjustment was insufficient to prevent sex ratio skews (Telemeco *et al.*, 2009). Instead, other components of nest-site choice might be more likely to compensate for climate change, either through microevolutionary change or phenotypic plasticity. For example, nesting phenology is behaviorally plastic in response to short-term climatic fluctuations; however, the heritability of nesting date is too low to allow this trait to respond to selection in painted turtles (Schwanz & Janzen, 2008). In another emydid turtle, nesting dates have become substantially earlier over the last decade, but the result has been a sex ratio skewed toward males because the earlier onset of nesting has allowed production of an additional clutch annually, and the final clutch produced in a year now experiences cool, male-producing temperatures during the thermosensitive period (Tucker *et al.*, 2008). Based on these studies of phenology, nesting date is not thought to be a strong compensatory mechanism for climate change in the species studied (Schwanz & Janzen, 2008; Mitchell & Janzen, 2010). Shade cover over the nest is another component of nest-site choice, and is known to affect sex ratio (Janzen, 1994b) and to shift in response to novel climatic conditions (Refsnider & Janzen, 2012) in the study species. Shade cover might be the component of nest-site choice most likely to compensate for climate change in freshwater turtles, and behavioral plasticity is at least one mechanism known to underlie choice of shade cover in painted turtles.

We found that deeper nests produced hatchlings that weighed less and were faster at both righting themselves and swimming than shallower nests. Although nest depth treatments did not differ statistically from each other in incubation regime, deep nests tended to be less variable than shallower nests (Fig. 4), a difference which may have been statistically significant if more nests in the deep treatment had remained after the flood event. As discussed above, flooding resulted in mortality of all but four nests in the deep treatment. The four remaining deep nests were similar in location, date of construction and shade cover, so the differences observed in offspring size and performance are not likely because of differences in nest microhabitat. However, maternal identity was unknown for these nests, so maternal or paternal effects on offspring performance cannot be excluded as an explanation for differences in offspring performance. In

contrast, a common-garden experiment (i.e. individuals from different populations housed under common environmental conditions) on the same species from five populations across a geographic range found that faster hatchlings were produced from nests that were more variable in daily temperature (Refsnider & Janzen, 2012). Other studies have also found differing effects of fluctuating temperatures on both sex ratio (e.g. Georges *et al.*, 1994; Neuwald & Valenzuela, 2011) and performance (e.g. Andrews, Mathies & Warner, 2000; Les, Paitz & Bowden, 2007; Du & Feng, 2008) of neonatal reptiles, even within the same population. For example, hatchling smooth softshell turtles (*Apalone mutica*) from an Iowa population were reported to swim faster as thermal variability during embryogenesis increased (Ashmore & Janzen, 2003), whereas a subsequent study of the same population found a less clear effect of such thermal variability on post-hatching swimming ability (Mullins & Janzen, 2006). Such diverse outcomes call into question the utility of generalizing about the effects of thermal variation experienced during embryonic development on the post-hatching performance of reptilian offspring. Results from our study suggest that the impacts of climatic warming on performance of hatching turtles are difficult to predict and likely will differ with latitude.

Although the depth of turtle nests varies geographically and tracks May temperature at our study site, results from

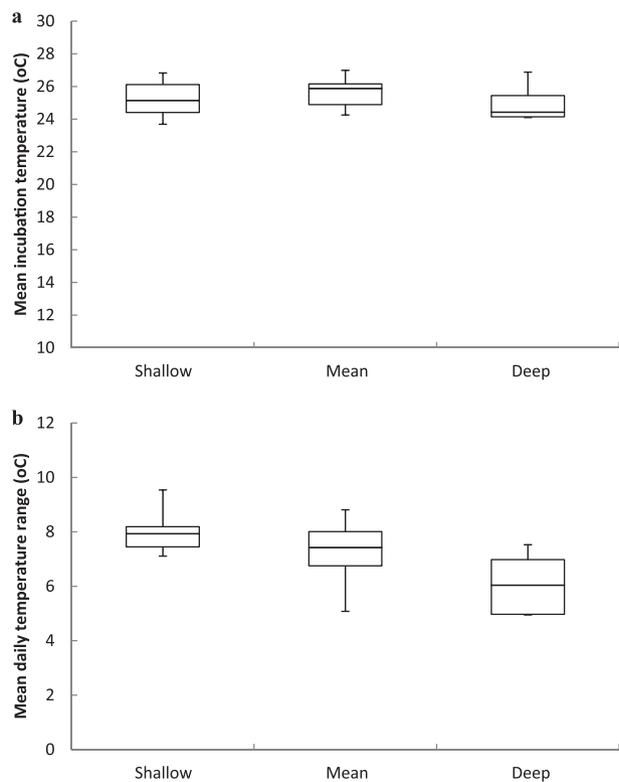


Figure 4 Mean incubation temperature (a) and mean daily temperature range throughout incubation (b) in painted turtle (*Chrysemys picta*) nests of three different depth treatments at Thomson Causeway Recreation Area, Carroll County, Illinois in 2010.

our study suggest that adjustment of nest depth is not likely to compensate for climate change in painted turtles. First, the amount by which nest depth would have to increase to affect sex ratio in the study population is 2 cm greater than, or two standard deviations from, the current population mean. Selection for a shift of this magnitude is likely relatively weak, and in some populations is probably biologically unfeasible without a simultaneous, substantial increase in female body size. Second, the proximate cues used by females to adjust nest depth may not reliably predict future incubation conditions. Finally, shade cover over the nest was a stronger predictor of sex ratio than nest depth. Therefore, shifts in components of nest-site choice other than nest depth, such as selection of shade cover over nest sites, may be more likely to allow this species to match incubation conditions to a changing climate. Importantly, variation among reptiles with TSD in whether nest depth adjustment affects sex ratio or responds to climatic variation (e.g. Vogt & Bull, 1982; Warner & Andrews, 2002; Morjan, 2003b; Doody *et al.*, 2006a; Mitchell *et al.*, 2008; Telemeco *et al.*, 2009) indicates that adjustment of nest depth does not have similar effects across taxa, and it should not be assumed that nest depth adjustment is a compensatory mechanism for climate change without evaluating its potential in particular species. In species where nest depth adjustment in response to inter-annual climatic variation has been observed, future research should focus on determining the relative importance of potential mechanisms underlying nest depth adjustment.

Acknowledgments

We thank T. Mitchell, H. Streby, J. Strickland and D. Warner for help with data collection; A. Bronikowski, P. Dixon, C. Kelly, E. Takle, H. Streby and two anonymous reviewers for helpful comments on the paper; and the US Army Corps of Engineers for access to the study site. This research was conducted in accordance with Institutional Animal Care and Use Committee protocol # 12-03-5570-J (Iowa State University). This study was funded by Sigma Xi Grants-in-aid-of-Research (to J. M. R.) and the National Science Foundation (DEB-064932 to F. J. J.).

References

- Andrews, R.M., Mathies, T. & Warner, D.A. (2000). Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetol. Monogr.* **14**, 420–431.
- Ashmore, G.M. & Janzen, F.J. (2003). Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* **134**, 182–188.
- Barnosky, A.D., Hadly, E.A., Bascompte, J., Berlow, E.L., Brown, J.H., Fortelius, M., Getz, W.M., Harte, J., Hastings, A., Marquet, P.A., Martinez, N.D., Mooers, A., Roopnarine, P., Vermeij, G., Williams, J.W., Gillespie, R., Kitzes, J., Marshall, C., Matzke, N., Mindell, D.P., Revilla, E. & Smith, A.B. (2012). Approaching a state shift in Earth's biosphere. *Nature* **486**, 52–58.
- Bergerard, J. (1972). Environmental and physiological control of sex determination and differentiation. *Annu. Rev. Entomol.* **17**, 57–74.
- Brooks, R.J., Bobyn, M.L., Galbraith, D.A., Layfield, J.A. & Nancekivell, E.G. (1991). Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). *Can. J. Zool.* **69**, 2667–2676.
- Brown, G.P. & Shine, R. (2004). Maternal nest-site choice and offspring fitness in a tropical snake (*Tropidonophis mairii*, Colubridae). *Ecology* **85**, 1627–1634.
- Bull, J.J. (1980). Sex determination in reptiles. *Q. Rev. Biol.* **55**, 3–21.
- Bulmer, M.G. & Bull, J.J. (1982). Models of polygenic sex determination and sex ratio control. *Evolution* **36**, 13–26.
- Burger, J. (1989). Incubation temperature has long-term effects on behaviour of young pine snakes (*Pituophis melanoleucus*). *Behav. Ecol. Sociobiol.* **24**, 201–207.
- Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M. (2006a). Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307–330.
- Doody, J.S., Guarino, E., Harlow, P., Corey, B. & Murray, G. (2006b). Quantifying nest site choice in reptiles using hemispherical photography and gap light analysis. *Herpetol. Rev.* **37**, 49–52.
- Du, W.-G. & Feng, J.-H. (2008). Phenotypic effects of thermal mean and fluctuations on embryonic development and hatchling traits a lacertid lizard, *Takydromus septentrionalis*. *J. Exp. Zool.* **309A**, 138–146.
- Ernst, C.H. (1971). Population dynamics and activity cycles of *Chrysemys picta* in southeastern Pennsylvania. *J. Herpetol.* **5**, 151–160.
- Ewert, M.A., Jackson, D.R. & Nelson, C.E. (1994). Patterns of temperature-dependent sex determination in turtles. *J. Exp. Zool.* **270**, 3–15.
- Frazer, G.W., Canham, C.D. & Lertzman, K.P. (1999). *Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs*. Simon Fraser University, Burnaby, British Columbia.
- Georges, A., Limpus, C. & Stoutjesdijk, R. (1994). Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *J. Exp. Zool.* **270**, 432–444.
- Hartman, C.A. & Oring, L.W. (2003). Orientation and microclimate of horned lark nests: the importance of shade. *Condor* **105**, 158–163.
- Janzen, F.J. (1994a). Climate change and temperature-dependent sex determination in reptiles. *Proc. Natl. Acad. Sci. USA* **91**, 7487–7490.

- Janzen, F.J. (1994b). Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. *Ecology* **75**, 1593–1599.
- Les, H.L., Paitz, R.T. & Bowden, R.M. (2007). Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. *J. Exp. Zool.* **307A**, 274–280.
- Leslie, A.J. & Spotila, J.R. (2001). Alien plant threatens Nile crocodile (*Crocodylus niloticus*) breeding in Lake St. Lucia, South Africa. *Biol. Conserv.* **98**, 347–355.
- Martin, T.E. & Roper, J.J. (1988). Nest predation and nest-site selection of a western population of the hermit thrush. *Condor* **90**, 51–57.
- McGaugh, S.E., Schwanz, L.E., Bowden, R.M., Gonzalez, J.E. & Janzen, F.J. (2010). Inheritance of nesting behaviour across natural environmental variation in a turtle with temperature-dependent sex determination. *Proc. R. Soc. B* **277**, 1219–1226.
- Miller, K. (1993). The improved performance of snapping turtles (*Chelydra serpentina*) hatched from eggs incubated on a wet substrate persists through the neonatal period. *J. Herpetol.* **27**, 228–233.
- Miller, K., Packard, G.C. & Packard, M.J. (1987). Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *J. Exp. Biol.* **127**, 401–412.
- Mitchell, N.J. & Janzen, F.J. (2010). Temperature-dependent sex determination and contemporary climate change. *Sex. Dev.* **4**, 129–140.
- Mitchell, N.J., Kearney, M.R., Nelson, N.J. & Porter, W.P. (2008). Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in the tuatara? *Proc. R. Soc. B* **275**, 2185–2193.
- Morjan, C.L. (2003a). How rapidly can maternal behavior affecting primary sex ratio evolve in a reptile with environmental sex determination? *Am. Nat.* **162**, 205–219.
- Morjan, C.L. (2003b). Variation in nesting patterns affecting nest temperatures in two populations of painted turtles (*Chrysemys picta*) with temperature-dependent sex determination. *Behav. Ecol. Sociobiol.* **53**, 254–261.
- Morjan, C.L. & Janzen, F.J. (2003). Nest temperature is not related to egg size in a turtle with temperature-dependent sex determination. *Copeia* **2003**, 366–372.
- Mullins, M.A. & Janzen, F.J. (2006). Phenotypic effects of thermal means and variances on smooth softshell turtle (*Apalone mutica*) embryos and hatchlings. *Herpetologica* **62**, 27–36.
- Neuwald, J.L. & Valenzuela, N. (2011). The lesser known challenge of climate change: thermal variance and sex-reversal in vertebrates with temperature-dependent sex determination. *PLoS ONE* **6**, e18117.
- Paukstis, G.L., Shuman, R.D. & Janzen, F.J. (1989). Supercooling and freeze tolerance in hatchling painted turtles (*Chrysemys picta*). *Can. J. Zool.* **67**, 1082–1084.
- Refsnider, J.M. (2012). *Effects of climate change on reptiles with temperature-dependent sex determination and potential adaptation via maternal nest-site choice*. PhD thesis, Iowa State University, Ames, Iowa.
- Refsnider, J.M. (2013). High thermal variance in naturally-incubated turtle nests produces faster offspring. *J. Ethol.* **31**, 85–93.
- Refsnider, J.M. & Janzen, F.J. (2010). Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annu. Rev. Ecol. Evol. Syst.* **41**, 39–57.
- Refsnider, J.M. & Janzen, F.J. (2012). Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination. *Biol. Conserv.* **152**, 90–95.
- Roosenburg, W.M. (1996). Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination? *Am. Zool.* **36**, 157–168.
- Schwanz, L.E. & Janzen, F.J. (2008). Climate change and temperature-dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiol. Biochem. Zool.* **81**, 826–834.
- Schwanz, L.E., Spencer, R.-J., Bowden, R.M. & Janzen, F.J. (2010). Climate and predation dominate juvenile and adult recruitment in a turtle with temperature-dependent sex determination. *Ecology* **91**, 3016–3026.
- Schwarzkopf, L. & Brooks, R.J. (1985). Sex determination in northern painted turtles: effects of incubation at constant and fluctuating temperatures. *Can. J. Zool.* **63**, 2543–2547.
- Shine, R. & Harlow, P.S. (1996). Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* **77**, 1808–1818.
- Shine, R., Madsen, T.R.L., Elphick, M.J. & Harlow, P.S. (1997). The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. *Ecology* **78**, 1713–1721.
- Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. & Miller, H.L. (2007). *Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge: Cambridge University Press.
- Stokes, D.L. & Boersma, P.D. (1998). Nest-site characteristics and reproductive success in Magellanic penguins (*Spheniscus magellanicus*). *Auk* **115**, 34–49.
- Telemeco, R.S., Elphick, M.J. & Shine, R. (2009). Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology* **90**, 17–22.
- Tiwari, M. & Bjorndal, K.A. (2000). Variation in morphology and reproduction in loggerheads, *Caretta caretta*, nesting in the United States, Brazil, and Greece. *Herpetologica* **56**, 343–356.
- Tucker, J.K., Dolan, C.R., Lamer, J.T. & Dustman, E.A. (2008). Climatic warming, sex ratios, and red-eared

- sliders (*Trachemys scripta elegans*) in Illinois. *Chelonian Conserv. Biol.* **7**, 60–69.
- Valenzuela, N. & Lance, V.A. (2004). *Temperature-dependent sex determination in vertebrates*. Washington, DC: Smithsonian Books.
- Van Damme, R., Bauwens, D., Braña, F. & Verheyen, R.F. (1992). Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* **48**, 220–228.
- Vogt, R.C. & Bull, J.J. (1982). Temperature controlled sex-determination in turtles: ecological and behavioral aspects. *Herpetologica* **38**, 156–164.
- Walsberg, G.E. & King, J.R. (1978). The heat budget of incubating mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) in Oregon. *Physiol. Zool.* **51**, 92–103.
- Warner, D.A. & Andrews, R.M. (2002). Nest-site selection in relation to temperature and moisture by the lizard *Sceloporus undulatus*. *Herpetologica* **58**, 399–407.
- Weisrock, D.W. & Janzen, F.J. (1999). Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Funct. Ecol.* **13**, 94–101.
- Wibbels, T., Bull, J.J. & Crews, D. (1994). Temperature-dependent sex determination: a mechanistic approach. *J. Exp. Zool.* **270**, 71–78.
- Wilson, K. & Hardy, I.C.W. (2002). Statistical analysis of sex ratios: an introduction. In *Sex ratios: concepts and research methods*: 47–92. Hardy, I.C.W. (Ed.). Cambridge: Cambridge University Press.
- Wolak, M.E., Gilchrist, G.W., Ruzicka, V.A., Nally, D.M. & Chambers, R.M. (2010). A contemporary, sex-limited change in body size of an estuarine turtle in response to commercial fishing. *Conserv. Biol.* **24**, 1268–1277.

COMMENTARY

Chelonians in a changing climate: can nest site selection prevent sex ratio skews?

D. Rödder & F. Ihlow

Section of Herpetology, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

CorrespondenceDennis Rödder, Section of Herpetology, Zoologisches Forschungsmuseum Alexander Koenig, 53113 Bonn, Germany
Email: d.roedder.zfmk@uni-bonn.de

doi:10.1111/acv.12079

Within the next decades, global environmental conditions are predicted to change dramatically with a magnitude exceeding changes experienced during the last millennia. In most areas, these changes will severely affect annual mean temperatures, especially in higher latitudes, and frequency of extreme weather events (Parry *et al.*, 2007). Global biodiversity will likely be affected across multiple scales, including potential extinctions, as has been suggested for the Monteverde golden toad (*Incilius periglenes* Anchukaitis & Evans, 2010) and the tuatara (*Sphenodon guentheri* Mitchell *et al.*, 2008), population declines and loss of genetic diversity (Bálint *et al.*, 2011; Habel *et al.*, 2011). Poikilothermic taxa, especially those species exhibiting temperature-dependent sex determination (TSD), are considered to be particularly vulnerable (Deutsch *et al.*, 2008; Hulin *et al.*, 2009; Huey, Losos & Moritz, 2010; Kallimanis, 2010; Mitchell & Janzen, 2010). Among these, taxa exhibiting FM-TSD, yielding males at higher temperatures, are suggested to be profoundly affected (Mitchell & Janzen, 2010), while species exhibiting inter-population variation in pivotal temperatures or transitional range of temperatures possess a larger potential to adapt and compensate increasing ambient temperatures (Hulin *et al.*, 2009).

The evolution of TSD in Chelonians has been subject to numerous scientific studies suggesting multiple independent evolution events (Janzen & Krenz, 2004; Valenzuela & Adams, 2011). By affecting gender ratios, TSD might be an evolutionary dead end in terms of climate change (Mitchell & Janzen, 2010), while also providing potential advantages under specific circumstances (Warner & Shine, 2008; Silber, Geisler & Bolortsetseg, 2011). An increasing number of scientific studies highlight the potential global chelonian diversity decline caused through climate change by screwing up sex ratios (e.g. Mitchell & Janzen, 2010; Ihlow *et al.*, 2012). Recent analyses of natural history traits regarding species exhibiting TSD suggest potential behavioral or phenological adaptations to compensate ambient fluctuations and therefore prevent skews in gender ratio (e.g. Doody *et al.*, 2006; Telemeco, Elphick & Shine, 2009; Refsnider & Janzen,

2010). However, previous studies revealed the magnitude of adjustments regarding nest depth that would be necessary to compensate for the effects of climate change to be unfeasible for some species (Mitchell *et al.*, 2008; Telemeco *et al.*, 2009).

In the current issue of *Animal Conservation*, Refsnider *et al.* (2013) test whether adjustments of nest depth may sufficiently compensate for gender ratio skews caused by rising ambient temperatures. The authors use nest depth measurements of more than 2300 nests of painted turtles *Chrysemys picta*, a frequently used model species in ecological studies (e.g. Valenzuela, 2009). Based on this dataset, they precisely describe the phenology of natural nests and experimentally manipulate nest depths to determine whether hatchlings emerging from shallow, medium or deep nests differ significantly in terms of fitness. Despite a rather low sample size of 21 experimentally modified nests included in their analyses, the authors detected statistically significant differences in offspring performance in 165 specimens among nest depth classes. Interestingly, they were not able to detect differences in incubation regime, survival, body size or sex ratio of hatchlings. A reasonable explanation is the physiological limitation of nest depth restricted by nesting females' body sizes, yielding a comparably narrow temperature range across all nest depth classes (table 1, Refsnider *et al.*, 2013). As temperature differences were only 1.2°C across nest depth classes, the potential of *C. picta* to compensate for the effects of increasing ambient temperatures related to climate change by adjusting nest depths appears to be rather limited, also suggesting low adaptation potential for other small-sized chelonian species exhibiting TSD. Previous studies revealed reptiles to select nest sites based on solar radiation or substrate temperature, which directly refers to canopy cover (Doody *et al.*, 2006). Refsnider *et al.* (2013) suggest shade cover to be a better predictor for gender ratios than nest depth. Despite fairly restricted sample sizes of eight nests designated as 'shallow' nine nests assigned to 'mean' and only four 'deep', this result is in concordance with previous studies suggesting shade

cover as an important factor to compensate thermal stress related to climate change (Refsnider & Janzen, 2012).

The study provides valuable insights in potential strategies of *C. picta* as a model organism to compensate for the effects of climate change. This kind of study is on the front line of research documenting the response of species facing environmental stress, which are urgently needed to estimate potential future losses of biodiversity. Future studies could also control for potential parental effects on hatchlings fitness by means of splitting clutches among treatments, consider a larger sample size and selecting artificial nesting sites in close proximity to reduce potential differences in humidity, precipitation and solar radiation. Documenting this type of fundamental ecological responses in a broad range of species will ultimately provide the baseline to understand interactions between biodiversity and climate change.

References

- Anchukaitis, K.J. & Evans, M.N. (2010). Tropical cloud forest climate variability and the demise of the Monteverde golden toad. *Proc. Natl. Acad. Sci. USA* **107**, 5036–5040.
- Bálint, M., Domisch, S., Engelhardt, C.H.M., Haase, P., Lehrian, S., Sauer, J., Theissinger, K., Pauls, S.U. & Nowak, C. (2011). Cryptic biodiversity loss linked to global climate change. *Nat. Clim. Chang.* **1**, 313–318.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. & Martin, P.R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* **105**, 6668–6672.
- Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M. (2006). Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307–330.
- Habel, J.C., Rödder, D., Schmitt, T. & Neve, G. (2011). Global warming will affect the genetic diversity and uniqueness of *Lycaena helle* populations. *Glob. Chang. Biol.* **17**, 194–205.
- Huey, R.B., Losos, J.B. & Moritz, C. (2010). Are lizards toast? *Science* **328**, 832–833.
- Hulin, V., Delmas, V., Girondot, M., Godfrey, M.H. & Guillon, J.-M. (2009). Temperature-dependent sex determination and global change: are some species at greater risk? *Oecologia* **160**, 493–506.
- Ihlow, F., Dambach, J., Engler, J.O., Flecks, M., Hartmann, T., Nekum, S., Rajaei, H. & Rödder, D. (2012). On the brink of extinction? How climate change may affect global chelonian species richness and distribution. *Glob. Chang. Biol.* **18**, 1520–1530.
- Janzen, F.J. & Krenz, J.G. (2004). Which was first, TSD or GSD? In *Temperature-dependent sex determination in vertebrates*: 121–130. Valenzuela, N. & Lance, V.A. (Eds). Washington: Smithsonian Institution Press.
- Kallimanis, A.S. (2010). Temperature dependent sex determination and climate change. *Oikos* **119**, 197–200.
- Mitchell, N.J. & Janzen, F.J. (2010). Temperature-dependent sex determination and contemporary climate change. *Sex. Dev.* **4**, 129–140.
- Mitchell, N.J., Kearney, M.R., Nelson, N.J. & Porter, W.P. (2008). Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? *Proc. Biol. Sci.* **275**, 2185–2193.
- Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. & Hanson, C.E. (Eds) (2007). *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II Contribution to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press.
- Refsnider, J.M. & Janzen, F.J. (2010). Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annu. Rev. Ecol. Evol. Syst.* **41**, 39–57.
- Refsnider, J.M. & Janzen, F.J. (2012). Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination. *Biol. Conserv.* **152**, 90–95.
- Refsnider, J.M., Bodensteiner, B.L., Reneker, J.L. & Janzen, F.J. (2013). Nest depth may not compensate for sex ratio skews caused by climate change in turtles. *Anim. Conserv.* **16**, 481–490.
- Silber, S., Geisler, J.H. & Bolortsetseg, M. (2011). Unexpected resilience of species with temperature-dependent sex determination at the Cretaceous-Palaeogene boundary. *Biol. Lett.* **7**, 295–298.
- Telemeco, R.S., Elphick, M.J. & Shine, R. (2009). Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology* **90**, 17–22.
- Valenzuela, N. (2009). The painted turtle, *Chrysemys picta*: a model system for vertebrate evolution, ecology, and human health. *Cold Spring Harb. Protoc.* doi: 10.1101/pdb.emo124
- Valenzuela, N. & Adams, D.C. (2011). Chromosome number and sex determination coevolve in turtles. *Evolution* **65**, 1808–1813.
- Warner, D.A. & Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* **451**, 566–568.

COMMENTARY

For reptiles with temperature-dependent sex determination, thermal variability may be as important as thermal averages

A. Georges

Institute for Applied Ecology, University of Canberra, Canberra, Australia

CorrespondenceArthur Georges, Institute for Applied Ecology, University of Canberra, Canberra, ACT 2601, Australia.
Email: georges@aerg.canberra.edu.au

doi:10.1111/acv.12080

Refsnider *et al.* (2013) express concerns that are widely held, that species with temperature-dependent sex determination (TSD) appear appallingly vulnerable to climate change. A relatively small shift in environmental temperature has the potential to dramatically shift offspring sex ratio, with less than one degree change in some species sufficient to move sex ratios from 100% of one sex to 100% of the other (Ewert, Jackson & Nelson, 1994; Young *et al.*, 2004). Furthermore, a number of studies have shown that species with TSD will be unable to accommodate predicted increases in global temperatures through phenotypic responses in timing of nesting or nest site selection (Schwanz & Janzen, 2008; Telemeco, Elphick & Shine, 2009; Mitchell & Janzen, 2010). The rate of change in climate is considered too rapid for effective evolutionary responses, and in any case, such evolutionary responses would be impeded by low, effective heritability in the pivotal temperature for sex determination (Bull, Vogt & Bulmer, 1982).

The enigma is that species with TSD have persisted through periods of climate experienced by the globe in the last 400 000 years equal to or exceeding that anticipated to occur under future climate change projections. The search is on for the mechanisms by which populations of TSD species can persist in the face of climate changes. Such understanding of how they have responded to past climate change can provide insight to how they might respond to future, human-induced climate change, and what we might be doing through habitat alteration and fragmentation to constrain their ability to respond.

Refsnider *et al.* (2013) explore the possibility that climate change may be accommodated by phenotypic or microevolutionary change in the depth at which female turtles nest. While nest depth has a relatively insignificant effect on mean temperatures experienced by the eggs, in the absence of movement of water or air and associated thermal load through the nest, it does alter the phase of the daily cycle in temperature and the magnitude of daily fluctuations. Sex of the offspring depends both on the mean temperature and on the magnitude of diel temperature fluctuations

(Georges, 1989). Indeed, it is possible to shift reptile sex ratios from 100% male to 100% female by altering the magnitude of diel fluctuations without altering the mean temperature at all (Georges, Limpus & Stoutjesdijk, 1994). When sex ratio does vary within a nest, it does so because the magnitude of diel fluctuations, not mean temperature, varies with depth (Georges, 1992). Thus, diel variation in nest temperature is as important as mean temperature in determining offspring sex ratios, and the magnitude of diel variation in temperature is strongly influenced by depth below the soil surface. For this reason, nest depth is a variable that could be manipulated by TSD species in response to climate change to maintain balance in offspring sex ratio. The predictions are that TSD species should seek warmer (exposed), more variable (shallower) thermal regimes in response to climate cooling or at higher latitudes or altitudes, and cooler (shaded), less variable (deeper) thermal regimes in response to climate warming or at lower latitudes or altitudes.

Climatic variation with latitude can be used as a surrogate for climate change in observational studies (Doody *et al.*, 2006; Doody, 2009), but Refsnider *et al.* (2013) instead overcome the complexities by undertaking an experimental approach – they manipulated nest depth using three treatments. The first was the typical core depth of 8.7 cm, the second shallow at 6.7 cm and the third deep at 10.7 cm. Mean shade cover was held relatively constant (48.0–50.2%). There was no significant difference in sex ratios produced under the three depth treatments. Indeed, even after manipulation of nest depth, shade cover remained a significant predictor of offspring sex ratio. The conclusion is that, for *Chrysemys picta* at least, manipulating nest depth is not likely to deliver benefits in the face of climate change. As variation in timing of nesting and nest site selection are also insufficient to deliver an adequate response, the species presumably faces range contraction through local extinction arising from gross imbalance in the population sex ratio.

More generally, there is a need for further manipulative studies of this kind on other species and across a broader

range of contexts. Refsnider *et al.* (2013) have studied a species that nests already in shaded conditions with modest diel variability in temperature. Nest temperatures in the European pond turtle, for example, vary by up to 18°C each day (Pieau, 1982). Such species have more scope to benefit from altering nest depth in response to climate change. Manipulative studies in the context of more pronounced drivers – stronger trends in thermal regime with depth than in the study of Refsnider *et al.* (2013) – may yield some additional insight to the benefit of nest depth manipulation by reptiles. Nevertheless, the study of Refsnider *et al.* (2013) provides a major advance in understanding the constraints faced by species with TSD in responding to climate change.

References

- Bull, J.J., Vogt, R.C. & Bulmer, M.G. (1982). Heritability of sex ratio in turtles with environmental sex determination. *Evolution* **36**, 333–341.
- Doody, J.S. (2009). Superficial lizards in cold climates: nest site choice along an elevational gradient. *Austral Ecol.* **34**, 773–779.
- Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M.W. (2006). Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307–330.
- Ewert, M.A., Jackson, D.R. & Nelson, C.E. (1994). Patterns of temperature-dependent sex determination in turtles. *J. Exp. Zool.* **270**, 3–15.
- Georges, A. (1989). Female turtles from hot nests: Is it amount of development or duration of incubation at high temperatures that matters? *Oecologia* **81**, 323–328.
- Georges, A. (1992). Thermal characteristics and sex determination in field nests of the pig-nosed turtle, *Carettochelys insculpta* (Chelonia: Carettochelydidae), from northern Australia. *Aust. J. Zool.* **40**, 511–521.
- Georges, A., Limpus, C.J. & Stoutjesdijk, R. (1994). Proportion of development at a temperature, not daily duration of exposure, determines sex in the marine turtle *Caretta caretta*. *J. Exp. Zool.* **270**, 432–444.
- Mitchell, N.J. & Janzen, F.J. (2010). Temperature-dependent sex determination and contemporary climate change. *Sex. Dev.* **4**, 129–140.
- Pieau, C. (1982). Modalities of the action of temperature on sexual differentiation in field-developing embryos of the European pond turtle *Emys orbicularis* (Emydidae). *J. Exp. Zool.* **220**, 353–360.
- Refsnider, J.M., Bodensteiner, B.L., Reneker, J.L. & Janzen, F.J. (2013). Nest depth may not compensate for sex ratio skews caused by climate change in turtles. *Anim. Conserv.* **16**, 481–490.
- Schwanz, L.E. & Janzen, F.J. (2008). Climate change and temperature-dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiol. Biochem. Zool.* **81**, 826–834.
- Telemeco, R.S., Elphick, M.J. & Shine, R. (2009). Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology* **90**, 17–22.
- Young, J.E., Georges, A., Doody, J.S., West, P.B. & Alderman, R.L. (2004). Pivotal range and thermosensitive period of the pig-nosed turtle, *Carettochelys insculpta* (Testudines: Carettochelydidae) from northern Australia. *Can. J. Zool.* **82**, 1251–1257.

COMMENTARY

Revealing the links between climate and demography for reptiles with environmental sex determination

L. E. Schwanz

Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Kensington, NSW, Australia

Correspondence

Lisa E. Schwanz, Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Kensington, NSW 2052, Australia

Email: l.schwanz@unsw.edu.au

doi:10.1111/acv.12081

For animals that deposit their eggs in terrestrial nests, the most vulnerable stage of life, embryonic development, is exposed to uncontrollable and sometimes unpredictable environmental conditions. Changes in the climate are known to alter temperatures within the nests of reptiles (Hays *et al.*, 2003; Schwanz *et al.*, 2010a). This may be a problem for developing embryos, as incubation temperatures have widespread effects on hatchling survival, morphology and performance (Deeming, 2004; Shine, 2004). In addition, for reptiles with temperature-dependent sex determination, development as a male or female is determined irreversibly during the egg stage (Janzen & Paukstis, 1991). Thus, it seems inevitable that climatic warming and its effects on early life stages will drive these animals to a demographic collapse.

However, the connection between climate and demography is not so straightforward for these species. First, compared with laboratory incubation studies, we know comparatively little about how conditions in natural nests influence phenotypes (see, e.g. Shine, Elphick & Harlow, 1997; Schwanz *et al.*, 2010a). Secondly, mean annual nest temperatures may not vary with annual climate if females alter their nesting behaviour as a function of climate (Doody *et al.*, 2006; Schwanz & Janzen, 2008; Telemeco, Elphick & Shine, 2009). Thirdly, the relationship between incubation temperature and phenotype (survival and sex) could vary with climate (Schwanz, Janzen & Proulx, 2010b) if, for example, yolks contain greater amounts of heat-shock proteins or hormones in extreme-temperature years (Janzen *et al.*, 1998; Bowden, Ewert & Nelson, 2000). Finally, if average nest temperatures warm under climate change and lead to reduced viability and biased sex ratios, selection on nesting behaviours or temperature dependence of phenotypes could lead to local evolutionary response (i.e. evolutionary adaptation).

Refsnider *et al.* (2013) tackle two of these issues in their paper on nest depth in painted turtles, a species with temperature-dependent sex determination. First, they consider how nest conditions in a natural setting influence

offspring phenotype. Secondly, they provide an analysis of whether variation in nest depth (i.e. a component of maternal nesting behaviour) can compensate for annual climatic variation. Moreover, they employ two under-represented methodological approaches – leveraging long-term data on a population (Schwanz *et al.*, 2009) and performing a manipulative experiment.

What the study finds is rather disheartening for animal conservation. Specifically, variation in nest depth, at least in this population of painted turtles, does not appear to be a likely candidate for behavioural compensation or microevolutionary response to climate change. Refsnider *et al.* (2013) show that nest depth, like the timing of nesting and the shading over the nest, is a component of maternal plasticity in nesting behaviour in painted turtles (Schwanz & Janzen, 2008; McGaugh *et al.*, 2010; Refsnider & Janzen, 2012). Mean nest depth was deeper in warmer years.

Changing nest depth does not seem to ameliorate the effects of climate change, however. In natural nests manipulated to be deep, average or shallow in their depth, nest depth had no statistically detectable effect on nest temperature (e.g. mean, maximum, daily range), nor did it influence nest sex ratio. Interestingly, despite the lack of detectable effect on nest temperature, nest depth treatment influenced offspring performance. Hatchlings from deep nests were faster at righting themselves when turned upside down and were faster in swimming trials. That performance, but not temperature, was influenced by nest depth treatment indicates that either: (1) very small differences in nest temperature can have profound effect on performance in this species or (2) variation in nest depth causes important variation in unmeasured nest traits, such as moisture, which, in turn, strongly influence hatchling performance.

Thanks to Herculean efforts in a few model species, a consistent picture is emerging regarding climate and its effects on hatchling sex ratios. First, across geographical scales, variation in nesting characteristics (nest timing and location) among populations of terrestrial and freshwater reptiles appears to be the main factor minimizing

geographic variation in hatchling sex ratios (Bull, Vogt & McCoy, 1982a; Doody *et al.*, 2006). Secondly, and in contrast to the geographical pattern, behavioural plasticity in nesting traits within a population across years appears to be inadequate to prevent climate-driven variation in nest temperatures and hatchling sex ratios (Schwanz & Janzen, 2008; Telemeco *et al.*, 2009; but see Refsnider & Janzen, 2012). This overall picture is compelling, but it comes from a few, well-studied species. Thus, one obvious recommendation for advancing the field is to invest in research on a greater number of species so that we can speak in generality.

Continued examination of model species with long-term data could also provide essential new insight. For example, are the patterns that we see across historical climatic variation predictive of what we can expect at extreme climates? Most notably, the sex ratio of hatchlings in painted turtles varies strongly with July air temperature, such that more males are produced in cool years and more females in warm years (Janzen, 1994; Schwanz *et al.*, 2010a). However, the extremely warm years do not produce all females (Schwanz *et al.*, 2010a). That is to say, at the extreme of warm temperatures (those relevant for climatic warming), behavioural or physiological plasticity may have a promising compensatory effect that they do not have at other temperatures.

Finally, long-term data on model species allow us to address the likelihood of local evolution. Heritability of traits related to reptile sex ratios has been measured in a handful of species, quantifying the potential of a population to respond to selection (Bull, Vogt & Bulmer, 1982b; Janzen, 1992; McGaugh *et al.*, 2010; Rhen *et al.*, 2011). However, we know almost nothing about selection on these traits. What is missing is information on (1) how variation in morphology and performance affects survival and reproductive success throughout an organism's life (Warner & Shine, 2008) and (2) how the strength of selection for survival compares to frequency-dependent selection on the sex ratio.

References

- Bowden, R.M., Ewert, M.A. & Nelson, C.E. (2000). Environmental sex determination in a reptile varies seasonally and with yolk hormones. *Proc. Biol. Sci.* **267**, 1745–1749.
- Bull, J.J., Vogt, R.C. & Bulmer, M.G. (1982b). Heritability of sex ratio in turtles with environmental sex determination. *Evolution* **36**, 333–341.
- Bull, J.J., Vogt, R.C. & McCoy, C.J. (1982a). Sex determining temperatures in turtles: a geographic comparison. *Evolution* **36**, 326–332.
- Deeming, D.C. (2004). Post-hatching phenotypic effects of incubation in reptiles. In *Reptilian incubation: Environment, evolution and behaviour*: 229–251. Deeming, D.C. (Ed.). Nottingham: Nottingham University Press.
- Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M. (2006). Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307–330.
- Hays, G.C., Broderick, A.C., Glen, F. & Godley, B.J. (2003). Climate chance and sea turtles: a 150-year reconstruction of incubation temperatures at a major marine turtle rookery. *Glob. Chang. Biol.* **9**, 642–646.
- Janzen, F.J. (1992). Heritable variation for sex ratio under environmental sex determination in the common snapping turtle (*Chelydra serpentina*). *Genetics* **131**, 155–161.
- Janzen, F.J. (1994). Climate change and temperature-dependent sex determination in reptiles. *Proc. Natl. Acad. Sci. USA* **91**, 7487–7490.
- Janzen, F.J. & Paukstis, G.L. (1991). Environmental sex determination in reptiles: ecology, evolution, and experimental design. *Q. Rev. Biol.* **66**, 149–179.
- Janzen, F.J., Wilson, M.E., Tucker, J.K. & Ford, S.P. (1998). Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. *Gen. Comp. Endocrinol.* **111**, 306–317.
- McGaugh, S., Schwanz, L.E., Bowden, R.M., Gonzalez, J.E. & Janzen, F.J. (2010). Inheritance of nesting behaviour across natural environmental variation in a turtle with temperature-dependent sex determination. *Proc. Biol. Sci.* **277**, 1219–1226.
- Refsnider, J.M. & Janzen, F.J. (2012). Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination. *Biol. Conserv.* **152**, 90–95.
- Refsnider, J.M., Bodensteiner, B.L., Reneker, J.L. & Janzen, F.J. (2013). Nest depth may not compensate for sex ratio skews caused by climate change in turtles. *Anim. Conserv.* **16**, 481–490.
- Rhen, T., Schroeder, A., Sakata, J.T., Huang, V. & Crews, D. (2011). Segregating variation for temperature-dependent sex determination in a lizard. *Heredity* **106**, 649–660.
- Schwanz, L.E. & Janzen, F.J. (2008). Climate change and temperature-dependent sex determination: can plasticity in maternal nesting behavior prevent extreme sex ratios? *Physiol. Biochem. Zool.* **81**, 826–834.
- Schwanz, L.E., Bowden, R.M., Spencer, R.-J. & Janzen, F.J. (2009). Nesting ecology and offspring recruitment in a long-lived turtle. *Ecology* **90**, 1709–1710.
- Schwanz, L.E., Spencer, R.-J., Bowden, R.M. & Janzen, F.J. (2010a). Climate and predation dominate early life-stages and adult recruitment in a turtle with temperature-dependent sex determination: insight from a long-term study. *Ecology* **91**, 3016–3026.
- Schwanz, L.E., Janzen, F.J. & Proulx, S.R. (2010b). Sex allocation based on relative and absolute condition. *Evolution* **64**, 1331–1345.
- Shine, R. (2004). Adaptive consequences of developmental plasticity. In *Reptilian incubation: Environment, evolution*

- and behaviour*: 187–210. Deeming, D.C. (Ed.). Nottingham: Nottingham University Press.
- Shine, R., Elphick, M.J. & Harlow, P.S. (1997). The influence of natural incubation environments on the phenotypic traits of hatchlings lizards. *Ecology* **78**, 2559–2568.
- Telemeco, R.S., Elphick, M.J. & Shine, R. (2009). Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology* **90**, 17–22.
- Warner, D.A. & Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* **451**, 566–569.

RESPONSE

Experimental field studies of species' responses to climate change: challenges and future directions

J. M. Refsnider^{1,2}, B. L. Bodensteiner¹, J. L. Reneker¹ & F. J. Janzen¹

¹ Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA, USA

² Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA, USA

Correspondence

Jeanine M. Refsnider, Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA

Email: refsnyder@berkeley.edu

doi:10.1111/acv.12084

The outcomes of climate change on biota are difficult to predict because they involve indirect effects of environmental changes on a multitude of organisms and occur via complex pathways. However, some thermally sensitive traits are directly impacted by climate and have demographic consequences for populations. One such trait is temperature-dependent sex determination (TSD), in which offspring sex is irreversibly determined by the temperature experienced by developing embryos; the extreme sensitivity of the TSD pathway means that a small change in environmental temperature could dramatically alter offspring sex ratio. Behavioral adjustment of maternal nest-site choice has been proposed as a mechanism allowing reptile populations to maintain even sex ratios despite climatic changes over evolutionary time. The rapid rate of human-induced climate change, however, may outpace the capacity of behavioral plasticity in long-lived species to adjust to a changing climate. We found that the adjustment in nest depth required to affect sex ratio in a model turtle species may be too great to keep pace with climate change, and therefore maternal adjustment of nest depth seems unlikely to compensate for sex ratio skews in small-bodied, freshwater turtles (Refsnider *et al.*, 2013).

One advantage of our experimental approach, which used natural nests under field conditions, was that nests experienced natural incubation regimes in terms of both mean and daily variation in temperature. As Georges (2013) points out, daily variation in nest temperature is as important as mean temperature in determining offspring sex ratios (Georges, 1989), and studies are increasingly finding effects of fluctuating temperatures during embryonic development on later life stages (e.g. Mullins & Janzen, 2006; Les, Paitz & Bowden, 2007; Refsnider, 2013). Moreover, whether temperature variation occurs near the upper or lower thermal limit differentially affects offspring survival (Les, Paitz & Bowden, 2009) and sex ratio (Neuwald & Valenzuela, 2011). These complications emphasize the importance of incorporating natural levels of temperature fluctuations in incubation experiments involving species with TSD.

Furthermore, understanding how juvenile performance (particularly as a result of incubation temperature during development) relates to fitness in adults would enhance our ability to predict the phenotypes of later life stages from the performance of earlier ones (Refsnider, 2013; Schwanz, 2013).

A disadvantage of conducting 'controlled' experiments under field conditions, as Rödder & Ihlow (2013) discuss, is that sample sizes are often small. In our study on how turtles may respond to climate change, sample sizes were halved after many nests were (perhaps ironically) destroyed by a flood, the likelihood of which also increases as climate change progresses (Milly *et al.*, 2002; Solomon *et al.*, 2007). Despite the difficulties of conducting experiments in the field, such an approach will provide valuable insight into species' responses to climate change, and similar studies on a variety of species will greatly advance our understanding of the interactions between biodiversity and climate change (Georges, 2013; Rödder & Ihlow, 2013; Schwanz, 2013).

An important area for continued research is the degree to which traits associated with nest-site choice, embryonic development and sex determination are genetically controlled. For example, studies that split clutches to parse out genetic variation due to parental effects (Rödder & Ihlow, 2013) will provide important insight into genetic versus environmental drivers of traits related to sex ratio. Heritability has been estimated for some such traits (McGaugh & Janzen, 2011; McGaugh *et al.*, 2011), but not yet for nest depth. In addition, researchers should strive to measure selection intensity on sex ratio-related traits (Schwanz, 2013). The strength of selection on traits related to TSD is likely to change as the climate continues to warm; moreover, selection strength on survival relative to sex ratio may also vary as climate change progresses (Schwanz, 2013). If we are to predict reptiles' response to climate change with any accuracy, we need a vastly improved understanding of the selective pressures posed by climate change and how they drive evolutionary responses of reptile populations.

References

- Georges, A. (1989). Female turtles from hot nests: is it amount of development or duration of incubation at high temperatures that matters? *Oecologia* **81**, 323–328.
- Georges, A. (2013). For reptiles with temperature-dependent sex determination, thermal variability may be as important as thermal averages. *Anim. Conserv.* **16**, 493–494.
- Les, H.L., Paitz, R.T. & Bowden, R.M. (2007). Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. *J. Exp. Zool. A* **307**, 274–280.
- Les, H.L., Paitz, R.T. & Bowden, R.M. (2009). Living at extremes: development at the edges of viable temperature under constant and fluctuating conditions. *Physiol. Biochem. Zool.* **82**, 105–112.
- McGaugh, S.E. & Janzen, F.J. (2011). Effective heritability of targets of sex-ratio selection under environmental sex determination. *J. Evol. Biol.* **24**, 784–794.
- McGaugh, S.E., Bowden, R.M., Kuo, C.-H. & Janzen, F.J. (2011). Field-measured heritability of the threshold for sex determination in a turtle with temperature-dependent sex determination. *Evol. Ecol. Res.* **13**, 75–90.
- Milly, P.C.D., Wetherald, R.T., Dunne, K.A. & Delworth, T.L. (2002). Increasing risk of great floods in a changing climate. *Nature* **415**, 514–517.
- Mullins, M.A. & Janzen, F.J. (2006). Phenotypic effects of thermal means and variances on smooth softshell turtle (*Apalone mutica*) embryos and hatchlings. *Herpetologica* **62**, 27–36.7.
- Neuwald, J.L. & Valenzuela, N. (2011). The lesser known challenge of climate change: thermal variance and sex-reversal in vertebrates with temperature-dependent sex determination. *PLoS ONE* **6**, e18117.
- Refsnider, J.M. (2013). High thermal variance in naturally-incubated turtle nests produces faster offspring. *J. Ethol.* **31**, 85–93.
- Refsnider, J.M., Bodensteiner, B.L., Reneker, J.L. & Janzen, F.J. (2013). Nest depth may not compensate for sex ratio skews caused by climate change in turtles. *Anim. Conserv.* **16**, 481–490.
- Rödder, D. & Ihlow, F. (2013). Chelonians in a changing climate: can nest site selection prevent sex ratio skews? *Anim. Conserv.* **16**, 491–492.
- Schwanz, L.E. (2013). Revealing the links between climate and demography for reptiles with environmental sex determination. *Anim. Conserv.* **16**, 495–497.
- Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. & Miller, H.L. (2007). *Contribution of Working Group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge: Cambridge University Press.